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(71) Applicant: PB DIAGNOSTIC SYSTEMS, INC. [US/US]; 151 University Avenue, Westwood, MA 02090-2399 (US).

(72) Inventors: BLACKWOOD, John, J. ; 14 Coolidge Avenue, Needham, MA 02192 (US). INBAR, Shai ; 6 Columbus Square, Boston, MA 02116 (US).

(74) Agent: MACCARONE, Gaetano, D.; PB Diagnostic Systems, Inc., 151 University Avenue, Westwood, MA 02090-2399 (US).

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(54) Title: ANALYTICAL ASSAY

(57) Abstract

A method for determining the amount of an analyte in a sample fluid utilizes an assay element which comprises at least one reagent layer. The assay method includes the steps of optically reading a signal producing species, e.g., a fluorescent label, a first time prior to depositing the sample fluid on the assay element and a second time, at the same wavelength and in the same location within the assay element, after the sample fluid has been applied to the assay element and the sample analyte has interacted with the reagent(s) present in the assay element. The first, or dry, reading is corrected for relative humidity and/or temperature variations.

ANALYTICAL ASSAY

BACKGROUND OF THE DISCLOSURE

The present invention relates to assays for the
determination of analytes in fluids and, more particularly, to a
method for correcting the signal obtained for variations caused by
5   relative humidity and/or temperature variations.

Many types of assay elements for the rapid analysis of
analytes present in biological fluids are known in the art. Of
particular interest are dry multilayer analytical elements to which the
sample, e.g., a drop of blood, serum or plasma, is applied and
10   allowed to migrate or diffuse to a reagent layer or layers. As a
result of the interaction between the analyte and the reagent(s)
present, a detectable change is brought about in the element
corresponding to the presence of the analyte in the sample. The
detectable change can be a color change which may be evaluated
15   visually or read spectrophotometrically such as with a densitometer.
In another scheme based on the presence of fluorescent-labeled

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biologically active species, a fluorescent output signal can be
generated and read spectrofluorometrically. Such assay elements
are of great interest because they can be adapted for use in
automated analytical instruments.

5   In the automated analytical instruments a sample of a
test fluid is typically provided in a sample cup and all of the assay
method steps including pipetting of a measured volume of the
sample onto an assay element, incubation and readout of the signal
obtained as a result of the interaction(s) between the reagent(s) and

10 the sample analyte are carried out automatically. The assay element
is typically transported from one station, e.g., the pipetting station,
to another, e.g., the optical read station, by a transport means such
as a rotating carousel to enable the test steps to be carried out
automatically. Further, in some instruments the pipetting and

15 optical read steps are carried out while the assay element is in a
temperature-controlled chamber. Such automated instruments can
be operated in the batch mode, that is, a plurality of assays for the
same analyte are carried out at the same time. Also, some
instruments are capable of being operated in a random-access mode,

20 that is, assays for a plurality of different analytes can be carried out
at the same time.

          Such automated analytical instruments are capable of
processing many assay elements rapidly and it is necessary to
achieve a very high level of precision for these assays. However,

25 imprecisions in the results obtained can be caused by a number of
factors. Commonly assigned, copending United States patent
application Serial No. 382,555, filed July 19, 1989 discloses and
claims an analytical method which utilizes dry multilayer assay

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elements. The method corrects for signal imprecisions caused by variations in reagent levels from assay element to assay element and other variations including those in instrument position response. Briefly, this method involves taking a first optical reading of the

5   assay element prior to delivering sample fluid to the element and, after the fluid has been dispensed and the requisite interaction between the sample analyte and the reagent(s) has taken place, taking a second optical reading. The ratio of the second signal to the first signal is taken and compared with that for known amounts

10 of the analyte to determine the amount of analyte in the fluid.

As the state of the art advances, however, additional difficulties may be encountered. For example, the optical signals obtained from various dry assay elements including multilayer assay elements can vary significantly depending upon the relative humidity

15 of the environment in which the instrument is located. Typically, these diagnostic assay elements are packaged in moisture impermeable materials and the relative humidity inside the package maintained at a very low level, e.g., about 10%, so as to prolong the shelf life of the elements. The assay elements should be

20 removed from the package just prior to use and inserted into the analytical instrument without being allowed to remain in the ambient environment for any extended length of time. However, it is apparent that the ambient environment will vary from location to location and also within a location over time. Therefore the relative

25 humidity within the instrument will vary.

Dealing with relative humidity and temperature considerations in random access analyzers involves still another level of difficulty since the individual assay elements may reside in the

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instrument for different periods of time before the dry optical reading
is taken. One approach to dealing with temperature considerations
in such analyzers is to allow the assay element to remain in a
temperature controlled chamber for the period of time necessary for

5   the element to equilibrate with the chamber temperature. A possible
approach to the relative humidity factor would be to take the dry
reading only after a particular assay element has been in the
instrument for a period of time sufficient to permit it to come to
equilibrium with the relative humidity of the environment and to

10   correct the reading based on the relative humidity as measured by a
sensor. Such procedures are not entirely satisfactory because
imposing such minimum time periods on the assay protocols would
restrict the throughput rate of the instrument and would not permit
the instrument to be operated in the most efficient manner.

15   It would be desirable therefore to have a method for
compensating for optical signal imprecisions caused by relative
humidity and/or temperature variations which offers a number of
optional choices based on the manner in which the operator desires
to practice the method. It would also be desirable to have such a

20   method which can be practiced so as not to adversely affect the
throughput rate of the analytical instrument. Further, it would be
desirable to have such a method which would permit a random
access automated analytical instrument to be operated in the most
efficient manner possible.

25   SUMMARY OF THE INVENTION

These and other objects and advantages are
accomplished in accordance with the invention by providing a
method for determining the amount of a component in a sample fluid

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such as plasma, serum, whole blood, buffer, etc. The assay method
is carried out with an assay element which includes at least one
reagent layer. The signal generating species in the assay element is
read optically a first time prior to delivering the sample fluid to the

5   element. Subsequently, after the sample fluid has been dispensed
to the assay element and the interaction between the sample analyte
and the reagent(s) present in the element has taken place, the signal
producing species is read optically a second time. The ratio of the
second optical signal to the first optical signal is taken and

10   compared with that for known amounts of the analyte to determine
the amount of analyte in the sample fluid.
             According to the method, the first, or dry, optical signal
is corrected for relative humidity and/or temperature variations.
Depending upon the manner in which the method is practiced, as

15   will be discussed in detail below herein, in correcting the signal the
method takes into consideration the temperature of the ambient
environment in which the instrument is located at the time of the
measurement and/or the relative humidity level of the environment
at the time of the measurement together with the length of time that

20   the assay element has been in the instrument.
             By correcting the dry optical signal in accordance with
the invention a number of choices are afforded to an operator since
it is possible to compensate for relative humidity and/or temperature
considerations based on the manner in which the operator desires to

25   practice the method. The method permits an automated analytical
instrument to be operated in a random access or batch mode
without any adverse effect on the throughput rate of the instrument.
Further, in one embodiment, the instrument can be operated in the

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most efficient manner possible since it is not necessary to wait any minimum period of time before taking the first optical reading nor is there any maximum period of time which can elapse before the first optical reading must be taken. In this embodiment processing of the

5   various different assay modules can be carried out essentially independently of such time considerations.

In another embodiment the first reading is taken immediately after inserting the assay element into the instrument in which case it is necessary to correct the optical signal only for the

10   ambient temperature since the assay element does not have time to undergo a change in relative humidity. Of course, in this embodiment it is necessary to insert the assay element into the instrument within a short period of time, e.g., up to about one minute, after removing it from the humidity controlled environment

15   in which it is packaged. According to another embodiment the assay element can be allowed to reside in a temperature controlled chamber of an instrument for a period of time sufficient for the element to equilibrate with the chamber temperature before the first reading is made. In this embodiment the optical signal is corrected

20   for relative humidity variations based on the relative humidity of the ambient environment and the period of time the assay element has been in the chamber.

In a preferred embodiment the assay method of the invention is carried out with an assay element which includes at

25   least one reagent layer and a light-blocking layer. The light-blocking layer provides an optical bound/free separation of the signal-generating species as a function of the amount of analyte in the sample fluid. The first and second optical readings are carried out

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by irradiating the same layer of the assay element in both instances at the same wavelength. In this preferred embodiment correction of the optical signal compensates for variations in reagent levels because of variations in reagent layer thicknesses from element to

5 element and for variations in the analytical instrument position response as well as relative humidity and/or temperature variations.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the invention as well as other objects and further features thereof, reference is made to the

10 following detailed description of various preferred embodiments thereof taken in conjunction with the accompanying drawings wherein: Fig. 1 is a partially schematic cross-sectional view of an assay element which can be utilized in the method of the invention;

15   Fig. 2A is a graphical illustration showing the dry reading optical signals obtained over a period of time in an 80% relative humidity experiment with one assay element;

          Fig. 2B is a graphical illustration showing the dry reading optical signals obtained over a period of time with assay

20 elements residing in a temperature controlled chamber at different relative humidity levels; and

          Fig. 3 is a graphical illustration showing the comparison of the experimental results (data points) shown in Fig. 2B to the results calculated (solid lines) from a mathematical equation

25 developed as a result of a proposed theoretical mechanism.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The assay elements which are utilized in the assay method of the invention may include any suitable signal-generating

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species. Any light radiation emitting or absorbing label, including a label which reacts with a reagent, which provides a detectable signal can be utilized as the signal-generating species. The label may be a fluorophore, a phosphor or a light absorbing material. The method

5   may be practiced with any "dry" assay element including those which are made up of only one reagent layer carried by a support layer, so called "multilayer" assay elements which have at least one reagent layer and at least one other layer such as a light-blocking layer, a layer for receiving a signal-generating species liberated from

10   another layer, etc.

        The assay method of the invention will be described in detail with respect to a preferred embodiment of an assay element which may be utilized therein. Referring now to Fig. 1 there is seen as assay element 10 which is a thin film multilayer element typically

15   having a thickness of about 0.1 mm and comprised of a transparent support 12 which carries in succession a reagent layer 14, a light-blocking layer 16 and an optional top coat layer 18 which may serve as a reagent layer, a filter layer such as for proteins, an anti-abrasion layer, etc. The reagent layer 14 is very thin, typically having a

20   thickness of about 0.025 mm and includes an immunocomplex of a binding partner for the analyte of interest and a conjugate of a labeled analyte (the same as the sample analyte, an analogue thereof or a structurally similar material which will bind to the binding partner). The binding partner, an antibody when the sample analyte

25   is an antigen, is immobilized in the reagent layer 14 by being covalently bound to the surface of the support layer 12, which may be of any appropriate material such as a polyester or a polystyrene, or to a matrix material or by being physically held by the matrix

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material. The matrix material may be a hydrophilic gel material such as gelatin, a polysaccharide, e.g., agarose, a derivatized polysaccharide, mixtures thereof, and the like. Light-blocking layer 16 may comprise any suitable material such as, for example, iron

5 oxide, titanium dioxide or the like dispersed in a binder material such as a polysaccharide. The optional topcoat layer 18 may comprise an anti-abrasion layer of a material such as a polysaccharide or preferably may include buffers, blocking and displacing agents, etc.

The assay element 10 may also include a layer or other

10 means (not shown) for distributing the sample fluid uniformly across the surface of the top layer of the element. Any suitable fluid distribution technique may be used including, for example, particulate layers, polymeric layers, fibrous layers, woven fabric layers and liquid transport systems which have been disclosed in the

15 art as being suitable for this purpose. Many such liquid distribution systems and materials for providing a uniform distribution of a fluid sample across the surface of an assay element are known in the art and therefore extensive discussion of such materials and systems is not required here. A particularly preferred fluid transport system is

20 that described in commonly assigned, copending application Serial No. 210,732, filed June 23, 1988. The distribution means, whether a layer of fibrous material, etc. or a liquid transport system is preferably relatively thick in comparison to reagent layer 14. In practice, the label which is present in reagent layer 14 is optically

25 read prior to applying the sample to the assay element by irradiating layer 14 with the appropriate electromagnetic radiation through transparent support layer 12 to obtain a first readout signal.

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According to the invention this first optical reading is corrected for relative humidity and/or temperature variations. As noted previously, the reading can be corrected for either temperature variations or relative humidity variations or both. Typically, the

5   reading is taken while the assay element resides in a temperature controlled chamber within an automated instrument. To correct for temperature variations the temperature of the ambient environment in which the instrument is located is used. The correction for relative humidity is based on the actual relative humidity level of that

10   environment at the time the measurement is taken and the period of time the assay element has been in the instrument. Accordingly, the ambient temperature and relative humidity and the amount of time the assay element is within the instrument are the variables which are taken into account in the correction of the dry reading.

15   To further aid those skilled in the art to understand and practice the invention, the proposed theoretical mechanism by which the dry reading is affected by relative humidity will be discussed. The proposed theoretical mechanism appears to explain the varying optical signals which were obtained from actual experiments as a

20   result of relative humidity and temperature variations. It should be understood, however, that the advantageous results which are provided by the method of the invention have been observed through extensive experimentation and therefore the proposed theoretical mechanism is not to be construed as limiting of the

25   invention.

Consider the situation where the assays are being carried out by means of an automated analytical element wherein a plurality, for example, seventeen assay elements are inserted into

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the temperature controlled chamber of the instrument and all the steps of the assay method performed while the assay elements reside therein. For purposes of illustration consider also that the assay elements are all for the determination of the same analyte, for
5   example, theophylline. An experiment was conducted by first calibrating the instrument at 20% relative humidity with a set of theophylline calibrators and storing the calibration curve. It should be noted here that this experiment was carried out at constant room temperature; the temperature dependence of the optical signals will
10 be discussed below. Subsequently, while in the 20% relative humidity environment, seventeen theophylline assay elements were loaded into the temperature controlled chamber and processed. The instrument batched the assay elements such that the first fourteen assays were completed, that is, a dry reading taken, the control
15 solution deposited and a second reading taken, prior to taking the first dry readings of the other three assay elements. Accordingly, assay elements 15-17 remained in the chamber for some period of time before the first dry readings of these elements were taken. The same theophylline control solution was applied to all the assay
20 elements. Thus, the dose result was expected to be the same (within precision) for all the elements. The dose results obtained are shown in Table I. It is seen that an "end" effect was observed with assay elements 15-17 because of the delay in their processing and that these assay elements gave higher dose results than the others
25 for the same sample fluid. At 20% relative humidity the mean of the readings for the last three assay elements was 11.7 mg/l compared to an overall mean of 11.0 mg/l.

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The instrument was then transferred to an 80% relative humidity environment and the procedure repeated with seventeen new assay elements using the same control theophylline solution. It is seen that the uncorrected dose results had a mean value of 13.8

5   mg/l and, further, that the dose results increase progressively with the period of time the particular assay element remained in the chamber before the first dry reading was taken. Accordingly, at constant temperature, the "end" effect was shown to be the result of a slow rise in the dry reading during incubation due to humidity.

10   To correct for this condition it was thought to be necessary to have the appropriate relationship between the dry reading, time, relative humidity and temperature and therefore the kinetics of the dry reading had to be ascertained. To study these kinetics a single assay element was loaded into the temperature

15   controlled chamber of the instrument in the 80% relative humidity environment and dry readings taken every thirty seconds beginning immediately. The dry readings initially decreased with time until a minimum was reached (about three minutes) and after a delay increased to a constant level when equilibrium was reached (about

20   twenty to twenty five minutes). The results are shown in Fig. 2A. The same phenomenon was observed at other relative humidity levels except that the final readings decrease with decreasing relative humidity as shown in Fig. 2B. The room temperature was the same during all these experiments (22°C).

25   Upon examination of the observed curves shown in Figs. 2A and 2B it was theorized that they are the result of two different phenomena, namely a temperature effect and a humidity effect. It is apparent that when the assay element is inserted into

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the temperature controlled chamber, its temperature begins to rise
from that of the ambient environment toward the chamber
temperaure, typically 37°C. Since the fluorescence of the dye used
as a label is inversely related to temperature, the intensity of the dry
5   reading will decrease as the temperature of the assay element
increases until an equilibrium is reached when the assay element
reaches the temperature of the chamber.

The equilibrium from lower temperature to an higher
temperature is a first order process of the type

$$A \xrightarrow{\;k_T\;} B$$

10   The mathematical expression for such kinetics is

$$dB/dt = k_T A \qquad B = B_o + Ae^{-k_T t}$$

where B_o is the final fluorescence intensity (at 37°C), A is the
difference in intensity between room temperature and 37°C, K_T is
the rate constant associated with the method and t is time.

For the specific case under consideration the equation
15   for the temperature effect is

$$D_{T(t)} = D_{37^o} + (D_{RT} - D_{37^o})e^{-k_T t}$$

where $D_{T(t)}$ is the dry reading as a function of time due to the
temperature effect, D_{37^o} is the dry reading at equilibrium (37°C) and
D_{RT} is the dry reading at room temperature, all of the above in a
constant relative humidity (the package relative humidity).

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In considering the humidity effect it was thought that a two part mechanism was responsible for the changes in the dry reading because of humidity variations. First, it was thought that the water vapor has to penetrate through the upper layers and reach

5   the reagent, or signal, layer (14 in Fig. 1) before the dry reading is affected since this layer is the only one irradiated to obtain the optical reading. Thus, a certain time delay was expected before the onset of the humidity effect.

Secondly, it was theorized that once the water vapor

10   reaches the signal layer a double kinetics follows: 1) water arriving at a certain rate to the layer; and 2) water reacting in a way as to cause a change in fluorescence. Although the exact cause of the change was not clear, the kinetics behavior of the change was expected to follow the pattern of:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

15   The mathematical expression for such kinetics is

$$dB/dt = k_1 A \qquad dC/dt = k_2 B$$

$$C_{(t)} = C_o + B[1 + (k_1 e^{-k_2 t} - k_2 e^{-k_1 t})/(k_2 - k_1)]$$

where $C_{(t)}$ is the dry reading as a function of time, C_o is the initial dry reading (at the package humidity), B is the difference between the dry reading at package humidity and room humidity, t is the time

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and K_1 and K_2 are the two rate constants associated with the process.

In the specific case being considered the equation can be written

$$D_{RH(t)} = D_{PK} + (D_{RH} - D_{PK})[1 + (k_1 e^{-k_2 t} - k_2 e^{-k_1 t})/(k_2 - k_1)]$$

5   where $D_{RH(t)}$ is the dry reading as a function of time as affected by the humidity change, D_{PK} is the dry reading at the package humidity and D_{RH} is the dry reading at room humidity.

To account for the time delay, t_D, the equation is modified somewhat to:

For $t > t_D$
$$D_{RH(t)} = D_{PK} + (D_{RH} - D_{PK})[1 + (k_1 e^{-k_2(t-t_D)} - k_2 e^{-k_1(t-t_D)})/(k_2 - k_1)]$$

For $t < t_D$
$$D_{RH(t)} = 0$$

10   The constants D_{PK} and $D_{37°}$ are in fact referring to the same quantity and are identical. This quantity can be labeled as D_o, the value that must be calculated for each assay element and use for the dry reading correction. The equation provides a signal which is initially constant and then rises until it reaches equilibrium.

15   The overall behavior of the assay elements is the sum of the two processes and is given by the combined equation

$$D_{RH,T(t)} = D_o + (D_{RT} - D_o)e^{-k_T t} +$$
$$(t > t_D)(D_{RH} - D_o)[1 + (k_1 e^{-k_2(t-t_D)} - k_2 e^{-k_1(t-t_D)})/(k_2 - k_1)]$$

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wherein D_o is the "basic" dry reading for the assay element (this is
the dry reading that would have been obtained at the chamber if
there were no humidity effect; this value must be calculated
according to the equation), D_{RT} is the initial dry reading at the
5   moment of inserting the assay element into the chamber; D_{RH} is the
final dry reading in the chamber after the assay element is
equilibrated for both the incubator temperature and the ambient
humidity; t_D is the delay time for the onset of the humidity effect; K_T
is the rate constant for the assay element thermal equilibrium in the
10   chamber; K_1 is the first rate constant for the effect of humidity on
the dry reading; K_2 is the second rate constant for the effect of
humidity on the dry reading; and $t > t_D$ is a logical expression that is
equal to 0 when $t < t_D$ and equal to 1 when $t > t_D$.

      The final kinetics pattern is the result of adding the two
15   curves according to the two phenomena. The resulting curve
closely resembles those obtained experimentally (Figs. 2A and 2B).

      Establishing the values for the equation parameters
involves fitting the experimental data to the equation described
above. Experimental data was obtained with many assay elements
20   for different analytes and from different production lots. Each set of
data produced a set of constants and after testing all the sets in the
experimental study, an average was taken.

      The experimental data can be fit to the equation by
means of a computer program. The program can find the equation
25   constant values for each of the experimental sets that give the best
fit between the experimental and the theoretical results. An
experimental data set is entered into the computer and the best fit is
found by a semi-interactive process. A program for providing the

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best fit can be based on a grid search where each of the constants
is tested over a range with many discreet values inside the range.
The best fit can be judged by the least square method. The ranges
of the search for each constant can be decided by initial estimates

5 made by the user.

The constants K_T, K_1 and K_2 are expected to be
independent of humidity and temperature and once their values are
established over many assay runs, an average can be calculated and
used in the equation. $D_{RT} - D_o$, which can be expressed as ΔD_T, is

10 expected to be humidity independent as long as the assay elements
are loaded into the instrument immediately after opening the
package (about one minute or less); it is expected to be room
temperature dependent. The dependence on temperature was
shown to be linear in the form of

$$\Delta D_T = A_T \cdot T + B_T$$

15 where A_T and B_T are constants which are found experimentally.

$D_{RH} - D_o$, which can be expressed at ΔD_{RH}, is
temperature independent and depends on humidity in a close to
linear fashion as established experimentally. In the final form of the
equation this expression is entered as a function of the measured

20 relative humidity,

$$t_D = C_1 \cdot RH^{-C_2}$$

The final equation containing only time (t), temperature
(T) and relative humidity (RH) as variables is

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$$D_{(t,RH,T)} = D_o + (A_T \cdot T + B_T)e^{-k_T t}$$

$$+ (t > C_1 \cdot RH - C_2) \cdot (A_r \cdot RH + B_r) \cdot [1 + (k_1 e^{-k_2(t - C_1 \cdot RH - C_2)}$$

$$- k_2 e^{-k_1(t - C_1 \cdot RH - C_2)})/(k_2 - k_1)]$$

where $D_{(t,RH,T)}$ is the dry reading at a time, t, a humidity, RH, and a room temperature, T.

The dry reading, D_o, to be used for normalization can be calculated from the equation

$$D_o = D_{(t,RH,T)} - (A_T \cdot T + B_T)e^{-k_T t}$$

$$- (t > C_1 \cdot RH - C_2) \cdot (A_r \cdot RH + B_r) \cdot [1 + (k_1 e^{-k_2(t - C_1 \cdot RH - C_2)}$$

$$- k_2 e^{-k_1(t - C_1 \cdot RH - C_2)})/(k_2 - k_1)]$$

5   Fig. 3 shows the comparison of the experimental results shown in Fig. 2B with the curves calculated from the equation using constants obtained with a computer program. The constants found were: $A_r = 9.7$; $B_r = -102$; $A_T = -75$; $B_T = 1950$; $C_1 = 94$; $C_2 = 0.85$; $K_t = 0.85$; $K_1 = 0.24$; $K_2 = 0.55$.

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It is seen that the fit between the experimental results and the theoretical equation is excellent and supports the proposed mechanism.

The equation in this form was used to correct the data
5   which illustrated the "end" effect. The corrected dose results are shown in Table I and clearly demonstrate that the "end" effect was eliminated by the method of the invention. For the 20% relative humidity results it can be seen that the mean value for assay elements 15-17 was 10.8 mg/l, the same as the overall corrected
10   mean value and the CV decreased to 1.72%. For the 80% relative humidity results the overall corrected mean value was 10.7 mg/l and the CV decreased to 5.31%.

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Table I

Assay	20% RH Theophylline (mg/l)		80% RH Theophylline (mg/l)	
Element	Uncorrected	Corrected	Uncorrected	Corrected
1	10.8	10.6	12.6	10.9
2	11.0	10.8	12.7	10.8
3	10.9	10.8	13.0	10.9
4	10.7	10.6	13.2	11.0
5	10.7	⋆10.6	12.9	10.6
6	10.8	10.7	12.7	10.3
7	10.8	10.7	12.8	10.1
8	10.8	10.4	13.3	10.5
9	10.5	10.8	14.3	11.3
10	10.9	11.0	13.7	10.7
11	11.0	10.9	12.1	9.1
12	10.9	11.3	13.6	10.4
13	11.3	10.8	15.7	11.3
14	10.8	10.8	15.8	11.3
15	11.7	10.7	16.1	11.6
16	11.6	10.9	15.2	10.8
17	11.9	10.9	14.9	10.6
Mean(mg/l)	11.0	10.8	13.8	10.7
SD	0.4	0.2	1.3	0.6
%CV	3.41	1.72	9.21	5.31

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In view of the foregoing it will be appreciated that by correcting the dry reading of an assay element in accordance with the invention using temperature, relative humidity and time as variables, the effect of relative humidity and temperature on the results obtained can be substantially eliminated. The relative humidity value at the time of the actual dry reading can be conveniently obtained by means of a relative humidity sensor arranged in the analytical instrument. It is preferred to locate the relative humidity sensor inside the instrument but outside the temperature controlled chamber in instances where fluid is dispensed to the assay elements while they reside in the chamber since the dimensions of the chamber and the frequency of dispensing of fluids can cause rapid, relatively large relative humidity variations within the chamber that are not followed as quickly by the test element. It has been found in such situations that sensing the relative humidity outside the chamber will provide the advantageous results according to the invention. The relative humidity sensor can be conveniently coupled to a microprocessor so as to transmit the relative humidity readings to the microprocessor continuously. Of course, the elapsed time that the assay element has been in the chamber is also conveniently continuously monitored by the microprocessor.

According to the method of the invention, after the dry reading is taken the sample fluid is then distributed across the surface of the assay element and the fluid diffuses throughout layers 14, 16 and 18 as well as any fluid distribution layer or liquid transport system present and an equilibrium is established. The analyte present in the sample will compete with the labeled analyte

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in reagent layer 14 for the available binding sites on the antibodies immobilized in layer 14, the labeled analyte being dissociated therefrom and replaced by the sample analyte in a ratio appropriately equal to the relative amounts of sample analyte and labeled analyte.

5   Thus, depending upon the amount of analyte in the sample, some percentage of the labeled analyte initially bound to the immobilized antibodies in layer 14 will be displaced therefrom and distributed throughout the remainder of the assay element. The amount of labeled analyte bound to the immobilized antibodies in reagent layer

10   14 at any time is inversely proportional to the amount of sample analyte.

A second readout signal is obtained by again irradiating reagent layer 14 through support layer 12 with the same electromagnetic radiation used in the first optical read step to obtain

15   a second signal which is inversely proportional to the amount of sample analyte, that is, the signal decreases as the amount of sample analyte increases. Since reagent layer 14 is relatively thin in comparison to the combined thickness of layers 16 and 18 together with that of any fluid distribution layer or liquid transport system

20   present and because light blocking layer 16 prevents any of the readout electromagnetic radiation from entering layer 18 or anything above it, the second signal obtained will be a function of the labeled analyte which is bound to the immobilized antibodies and a small percentage of the free labeled analyte which is distributed

25   throughout the remainder of the assay element. In a preferred embodiment the ratio of the thickness of reagent layer 14 to the combined thickness of the light-blocking layer and the remainder of the assay element is from about 1:20 to about 1:100 or more.

The ratio of the second signal to the first signal is taken and compared with that for known amounts of the analyte to determine the amount of analyte in the sample fluid. The ratio may be used as obtained or it may be multiplied by some constant,

5   dependent upon the particular assay, to provide a signal which falls in some desired range.

It will be appreciated that the method of the invention can be practiced according to a number of embodiments thereof. In one, the method can be practiced such that it is necessary to

10   correct for temperature only by removing the assay element from a relative humidity controlled environment, for example, its package, inserting it into the instrument within a very short period of time, for example, a minute or less and taking the first dry reading immediately. In another embodiment the method can be practiced in

15   a manner such that it is necessary to correct only for relative humidity by allowing the assay element to remain in a temperature controlled chamber for the period of time required for the element to equilibrate with the chamber temperature. In still another embodiment the method can be practiced without regard to any

20   such minimum or maximum time considerations and the optical signal corrected for relative humidity and temperature. According to this embodiment the method of the invention can be carried out in the most efficient manner permitted by the particular instrumentation used to carry out the assay. Since it is not

25   necessary to wait for any minimum length of time to take the dry reading and, on the other hand, the dry reading can be taken after any amount of time has elapsed after the assay element has been inserted into the temperature controlled chamber, a plurality of assay

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elements may be processed simultaneously and the sequence of
steps practiced with each assay element determined according only
to factors which result in the most efficient operation of the
instrumentation. While it will be appreciated that this is so for batch
5 processing of a plurality of assay elements for testing for the same
analyte, the method of the invention is particularly advantageous for
random access processing, that is, where assay elements for testing
for different analytes are processed simultaneously and, further,
individual ones of the assay elements are removed from the
10 instrument when the particular assay is complete and others inserted
in their places while still others remain in the chamber because the
particular assay is not yet finished. This capability of an instrument
to handle a plurality of different assay elements for different
analytes having varying assay protocols, permits the instrument to
15 be operated in a very efficient mode and advantageously allows the
throughput of the instrument to be maximized. In accordance with
the practice of the method, variations in the instrument position
response and in the thickness of the reagent layer(s) from assay
element to assay element as well as relative humidity and/or
20 temperature variations can be compensated for and significantly
better precision obtained.

      The invention will now be described further in detail
with respect to specific preferred embodiments by way of examples,
it being understood that these are intended to be illustrative only and
25 the invention is not limited to the materials, procedures, etc. recited
therein.

EXAMPLE I

An assay element was prepared comprising a
transparent polyethylene terephthalate support having coated
thereon in succession:

5   1. a reagent layer comprising about 500 mg/m^2 of a
3:1 mixture of agarose and glyoxyl agarose; about 72 mg/m^2 of bis
tris propane buffer; about 10 mg/m^2 of an antibody raised against
theophylline; and about 0.07 mg/m^2 of a fluorescent labeled
theophylline conjugate represented by the formula
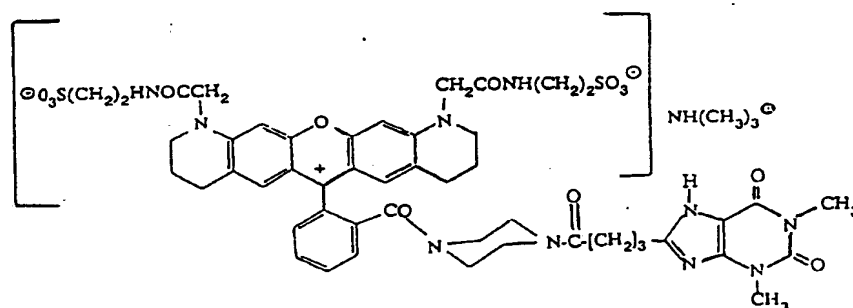
10   2. a light-blocking layer comprising about 6000 mg/m^2
of iron oxide, about 2000 mg/m^2 of agarose and about 50.4 mg/m^2
of 2'-morpholino ethane sulfonic acid (pH 5.7); and

          3. a topcoat layer comprising about 2000 mg/m^2 of
agarose.

15   Seventeen such assay elements were inserted one after
the other into a temperature controlled chamber (37°C) of an
automated laboratory instrument. First and second readings of the

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assay elements were taken by irradiating the assay element through
the transparent support with 550 nm excitation energy and the
fluorescent emissions measured at 580 nm. The instrument
processed the assay elements in the manner described above herein.

5   Similar experiments were carried out at 20% and 80% relative
humidity, respectively. The uncorrected results, together with those
obtained by correcting these results in accordance with the
invention, are shown in Table I. Although the invention has been
described with respect to specific preferred embodiments, it is not

10 intended to be limited thereto but rather those skilled in the art will
recognize that variations and modification may be made therein
which are within the spirit of the invention and the scope of the
appended claims.

What is claimed is:

      1. A method for determining an analyte in a sample fluid comprising

          (a) irradiating a region of an assay element including a signal generating species with electromagnetic radiation to obtain a
5   first optical signal prior to applying any sample fluid to said assay element;

          (b) correcting said first optical signal for relative humidity and/or temperature variations;

          (c) applying a sample fluid to said assay element;

10   (d) irradiating said assay element in the same region and with the same electromagnetic radiation utilized in (a) to obtain a second optical reading; and

          (e) determining the amount of analyte in said sample fluid as a function of said corrected first optical reading and said
15   second optical reading.

      2. The method as defined in claim 1 wherein said assay element comprises (i) a light-blocking layer which is permeable to said sample fluid and (ii) a reagent layer, said signal generating species residing in said reagent layer; and step (e) comprises taking
20   the ratio of said second optical signal to said corrected first optical signal and comparing said ratio with those obtained for known amounts of said analyte.

      3. The method as defined in claim 2 wherein said assay element further incudes a support which is transparent to said
25   electromagnetic radiation.

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4. The method as defined in claim 2 wherein said reagent layer includes an immobilized binding partner for said analyte and said signal generating species comprises a conjugate of a label bound to a moiety which is capable of binding to said binding
5 partner.

5. The method as defined in claim 4 wherein said moiety bound to said label is said analyte or an analogue thereof.

6. The method as defined in claim 4 wherein said label is fluorescent.

10   7. The method as defined in claim 1 wherein step (b) comprises correcting said first optical signal for relative humidity and temperature variations.

8. The method as defined in claim 1 wherein step (b) comprises correcting said first optical signal for relative humidity
15 variations.

9. The method as defined in claim 1 wherein step (b) comprises correcting said first optical signal for temperature variations.

10. A method for processing a plurality of assay
20 elements in an analytical instrument comprising

(a) inserting a plurality of assay elements into a temperature controlled chamber of an analytical instrument;

(b) irradiating at least one of said assay elements in a region thereof which includes a sample generating species with
25 electromagnetic radiation to obtain a first optical signal for said assay element prior to applying any sample fluid to said element;

(c) correcting said first optical signal for said assay element for relative humidity and/or temperature variations;

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(d) applying sample fluid to said assay element;

(e) irradiating said assay element in the same region and with the same electromagnetic radiation utilized in (b) to obtain a second optical reading for said element; and

5   (f) determining the amount of analyte in said sample fluid as a function of said corrected first optical reading and said second optical reading.

11. The method as defined in claim 10 wherein step (c) comprises correcting said first optical signal for each said assay

10   element for relative humidity variations.

12. The method as defined in claim 10 wherein step (c) comprises correcting said first optical signal for each said assay element for temperature variations.

13. The method as defined in claim 10 wherein step

15   (c) comprises correcting said first optical signal for relative humidity and temperature variations.

14. The method as defined in claim 10 wherein said assay element comprises (i) a light-blocking layer which is permeable to said sample fluid and (ii) a reagent layer, said signal generating

20   species residing in said reagent layer, and step (f) comprises taking the ratio of said second optical signal to said corrected first optical signal and comparing said ratio with those obtained for known amounts of said analyte.

15. The method as defined in claim 14 wherein said

25   reagent layer includes an immobilized binding partner for said analyte and said signal generating species comprises a conjugate of a label bound to a moiety which is capable of binding to said binding partner.

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16. The method as defined in claim 15 wherein said moiety bound to said label is said analyte or an analogue thereof.

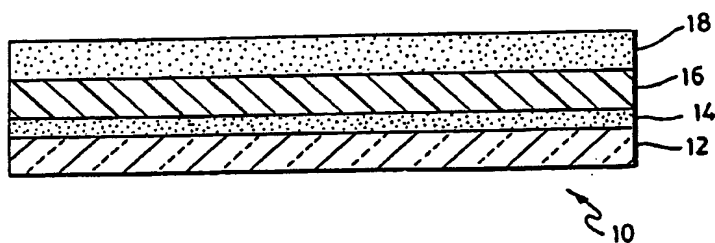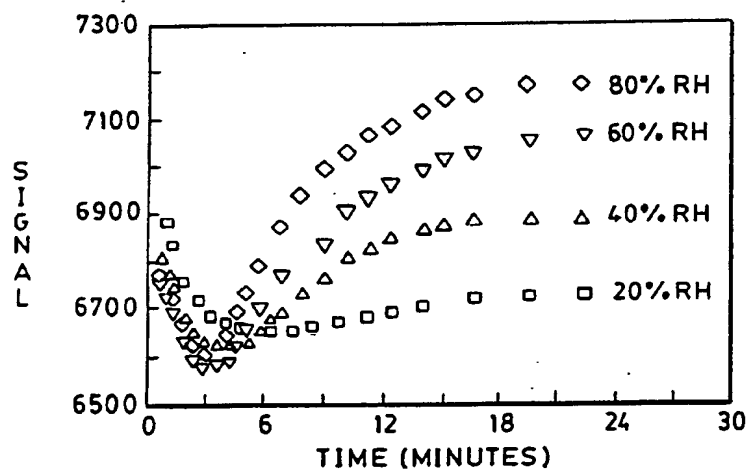17. The method as defined in claim 16 wherein said label is fluorescent.
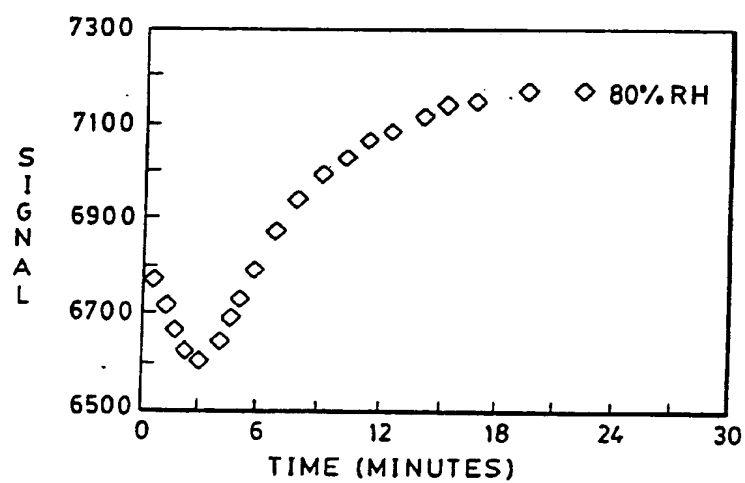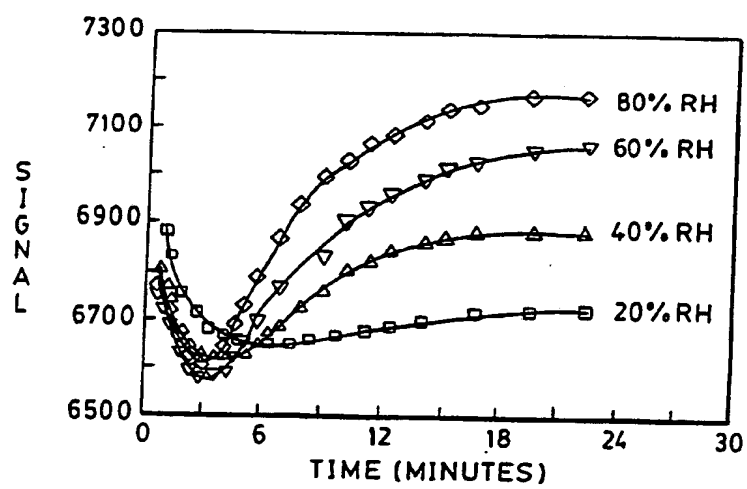
-1/3-

FIG. I

FIG. 2B

FIG. 2A

FIG. 3

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N21/86 ; G01N35/00 ; G01N33/543

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	CLINICAL CHEMISTRY. vol. 35, no. 9, September 1989, WINSTON US pages 1865 - 1868; G. GRENNER ET AL.: 'Multilayer fluorescent immunoassy technique' see page 1865, column 1, paragraph 1 see page 1865, column 2, paragraph 3 see page 1868, column 2, paragraph 2 ---	2,14
A	FR,A,2 282 641 (DAMON CORP.) March 19, 1976 see page 7, line 12 - line 27; figure 7 see page 8, line 8 - line 16 see page 12, line 15 - line 24 see page 13, line 2 - line 16 see page 17, line 6 - line 25 see page 17, line 37 - page 18, line 6 see page 24, line 19 - page 25, line 20 see page 26, line 20 - line 35 --- -/--	1,10

* Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 AUGUST 1991	13. 09. 91

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	ZINNGREBE U.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,353 590 (ABOTT) February 7, 1990 see page 18, line 56 - page 19, line 30 see page 20, line 32 - line 38 see page 20, line 52 - page 21, line 6 ---	1,10

US 9102091
SA   47264

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28/08/91

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2282641	19-03-76	CA-A- 1049386	27-02-79
		DE-A- 2536886	04-03-76
		GB-A- 1522965	31-08-78
		JP-A- 59166844	20-09-84
		JP-C- 1334368	28-08-86
		JP-A- 51055292	14-05-76
		JP-B- 59039698	26-09-84
EP-A-353590	07-02-90	AU-A- 3884889	08-02-90
		JP-A- 2075954	15-03-90